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STUDIES IN THE PHYSIOLOGY OF THE FUNGI

VI. THE RELATION OF BACTERIA TO CELLULOSE FERMENTATION INDUCED BY FUNGI, WITH SPECIAL REFERENCE TO THE DECAY OF WOOD

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It is a matter of common observation and easy demonstration that bacteria are invariably present when wood decays under ordinary conditions. That a majority of these forms would be the ordinary air and soil saprophytes is only to be expected, yet the presence of cellulose-dissolving forms is not precluded. The purpose of this investigation is to determine whether or not cellulose-dissolving bacteria are habitually present on decaying wood under natural conditions and their influence, if any, on the rate of decay, also to determine whether or not the ordinary saprophytic forms, so universally present, in any way affect the cellulose-dissolving proclivities of the fungus actually causing the decay, by changing the reaction of the substratum so that it may be more or less favorable for optimum growth.

Due to the facts that all wood used in this work was first sterilized by autoclaving and that the theories regarding the effect of autoclaving wood are somewhat diverse, it was found desirable, if not expedient, to determine first whether or not this process of sterilizing wood produced new conditions, so that the results would not be comparable to those which might obtain under natural conditions.

SURVEY OF LITERATURE

EFFECTS OF AUTOCLAVING WOOD

The different viewpoints in regard to the effects of sterilization upon woody tissues are indicated by the works of Potter ('04) and Spaulding ('06). The earlier workers in this field were primarily interested in the determination of the chemical compounds present in woody tissues. Thus, Singer ('82) extracted four distinct substances by means of boiling water: vanillin, a substance giving the reactions of coniferin, a water-soluble gum, and also a substance giving a yellow color with hydrochloric acid. Van Wisselingh ('98) found that after treating slices of *Beta vulgaris* with distilled water at 125° C. for six hours a wall of pure cellulose remained, while the pectin substances were decomposed and dissolved. Even at a temperature of 100° C. the tendency for water to decompose and dissolve the cell wall was noted.

Potter ('04) tried the effect of hot water on different woods, *Quercus*, *Ulmus*, *Alnus*, *Aesculus*, and others, and found that steaming had a decided effect on the cell wall. It was shown that the action of boiling water caused a delignification of the xylem. The watery extract obtained from sawdust and fragments of wood when treated in this manner contained a substance which reacts to the lignin tests. Still further, it was shown that cold water, operating for a longer period of time, had a similar power of extracting from the xylem a substance which reacts with phloroglucin and thallin sulphate, and thus by continued soaking in water wood undergoes a partial delignification. If this be true, then autoclaving, even for the time required for sterilization, may have a marked effect on the wood when used for experimental purposes with reference to decay induced by fungi.

Rose and Lisse ('17) have made chemical analyses of sound Douglas fir and of the same wood in various stages of decay. Their results showed a rapid decrease in the percentage of cellulose as decay proceeded, while the lignin seemed to be far more resistant. They do not state what fungus caused the decay of the wood upon which they worked, and for that

reason their results are far less valuable and can in no way be taken as general. It has been shown by von Schrenk ('00) that even on the same kind of wood two fungi may act in an entirely different manner with respect to which portion of the woody tissue is utilized. Thus, *Polyporus juniperinus* growing on the wood of *Juniperus virginiana* caused a delignification in the first stages of decay and the remaining wood fiber was completely reduced to cellulose. *Polyporus carneus*, on the other hand, almost entirely removed the cellulose when growing on the same wood.

Spaulding ('06) differs to some extent from the views expressed by Potter. Steaming tests were made with a variety of woods, thin sections being placed in the Arnold sterilizer or the autoclave and the effects noted by micro-chemical reactions. Spaulding obtained slight delignification in *Sassafras Sassafras* after fifteen hours of boiling, and in *Picea rubens* after seven hours in the autoclave or with interrupted boiling after a total of twenty-seven hours. With these two exceptions forty hours of boiling seemed to have no effect upon the lignin of the sections. In other tests, using the autoclave at a temperature of about 120° C., it was found that many woods gave evidences of delignification after fifteen hours of treatment, and nearly all after eighteen hours.

Spaulding, however, does not consider these effects important with reference to experimental work with the wood-decaying fungi, since it is claimed even the very thin sections had to be subjected to the action of boiling water for relatively long intervals before any decided effect could be detected. In discussing the effects of boiling water on finely divided wood, however, he admits that lignin is extracted in sufficient quantity for lignin reactions to be obtained in the filtrate from the extracted material. The effect of autoclaving thin shavings for one hour was also tested by Zeller ('17). It was found that the sections were not delignified to such an extent that by staining with zinc chloriodid any change could be detected, although the water in which the shavings had been boiled gave a very faint pink color with phloroglucin and hydrochloric acid.

THE RELATION OF BACTERIA TO CELLULOSE FERMENTATION

The fact that certain microorganisms have the power to dissolve cellulose has been known for some time. The actual determination of the forms involved and a better understanding of the process resulted from more recent investigations.

Mitscherlich ('50) first noted that the cellulose in the cell walls of slices of potato was dissolved when the slices were immersed in water and kept in a warm place for several days. A few years later attention was called by Haubner to the fact that it was impossible to recover more than 50 per cent of the crude fiber fed to ruminants. This observation was soon confirmed by numerous other workers, but the agent causing this reduction was unknown at that time. It has been recently demonstrated by Ellenberger ('15) and others that cellulose-dissolving ferments could not be extracted from the intestines of herbivorous animals, nor could any such ferments be obtained from the walls of the stomach or any of the secretive glands. The fungous flora of the intestines, however, showed numerous cellulose-dissolving organisms.

Some ten years after Haubner's discovery Trécul ('65) described a new genus, *Amylobacter*, which he found in connection with macerated plant tissues. Following this work numerous other workers described what they thought to be new forms. Thus we find *Bacterium navicula* of Reinke and Berthold ('79), the hydrogen and methane ferments of Omelianski, *Clostridium polymyxa*, *Vibrio rugula*, *Clostridium butyricum* of Prazmowski ('80), and others, all having the power to dissolve cellulose. At first there was considerable confusion regarding the validity of these forms. Van Tieghem ('77), working on Trécul's *Amylobacter*, found it to be a motile rod and called it *Bacillus amylobacter*. Prazmowski ('80) regarded his *Clostridium butyricum* as synonymous with *Vibrio butyrique* Pasteur, *Amylobacter* Trécul, *Bacillus amylobacter* Van Tieghem, and *Bacterium navicula* Reinke and Berthold. The work of Omelianski ('95, '97, '02, '04) greatly contributed to a clearer conception of the causative organisms in cellulose fermentation and a better understanding of the processes involved.

It is of special interest to note that *Bacillus amylobacter*, as shown by Van Tieghem ('77), does not dissolve the cellulose from all plants, and also the fact, as indicated by Omelianski, that with the forms worked on by him the power to dissolve cellulose decreased as the age of the cultures increased. McBeth and Scales ('13) found that cellulose-dissolving bacteria are universally present in cultivated soils.

That even the ordinary saprophytes may, under some conditions, affect some of the components of the cell wall, is shown by van Hall ('02). He found that *Bacillus subtilis* and *B. vulgar-gatus* could produce a rot on potatoes, turnips, and various nuts at a temperature of from 37 to 42° C. and that the middle lamella was dissolved. Potter ('04) also found that in the decoction made by boiling the alburnum of oak wood *Bacillus subtilis* could grow and utilize the extracted substance (lignin).

EXPERIMENTAL WORK

THE EFFECTS OF AUTOCLAVING WOOD

Color changes induced in Douglas fir and western hemlock.—Equal amounts, 2.000 gms., of fine Douglas fir sawdust were placed in 125-cc. Erlenmeyer flasks and 25 cc. of distilled water were added to each. The flasks and contents were then weighed to the closest 0.1 gram preparatory to autoclaving, so that if any change in weight took place during autoclaving the flask could be restored to its original weight by the addition of distilled water, any change in the intensity of color then not being due to an increased concentration of the contents. The same methods were followed with western hemlock sawdust.

Some flasks and contents were not autoclaved; others were autoclaved for increasing intervals from forty-five minutes to twenty-one hours and the change of color noted. The colors are those of Ridgway ('12).

In both cases it is evident that a decided darkening occurs as the length of autoclaving increases. For the purposes of sterilization twenty hours of autoclaving are not necessary, yet there is a marked change in color caused by forty-five minutes of this treatment. The exact nature of these changes

TABLE I
COLOR CHANGES INDUCED BY AUTOCLAVING DOUGLAS FIR AND WESTERN HEMLOCK SAWDUST FOR VARIOUS INTERVALS

Interval of autoclaving	Color	
	Douglas fir	Western hemlock
Control (not autoclaved)		
45 minutes.....	Antimony-yellow	Cinnamon-buff
10 hours.....	Cinnamon-buff	Sayal-brown
20 hours.....	Cinnamon	Snuff-brown
	Mikado-brown	Natural brown

is not entirely known but it is quite evident that some changes must occur due to the prolonged heating.

Relative amounts of reducing substances in the aqueous extracts after autoclaving Douglas fir sawdust.—Into each of fifteen thoroughly cleaned 125-cc. Erlenmeyer flasks equal amounts, 2.000 gms., of fine Douglas fir sawdust were placed. The sawdust was prepared from the heart-wood in the following manner: Large pieces were sawed in the direction of the grain, resulting in a rather fine to coarse sawdust. This was then ground in a bone grinder until a comparatively fine mixture was obtained. The sawdust was dried to constant weight in a hot-air oven at a temperature of 85° C. and then allowed to stand uncovered in the laboratory for twenty-four hours. It was assumed that a fairly constant weight would be attained during that time, the amount of moisture taken up depending on the relative humidity of the air and the temperature. This procedure was followed rather than that of taking equal amounts of thoroughly dry sawdust, since it was found that a considerable fluctuation in weight took place during the process of weighing due to absorption of atmospheric moisture.

To each of the flasks 25 cc. of distilled water were added, and each flask and contents weighed, as before, to the nearest 0.1 gm., in order that the flasks might be restored to their original weight after autoclaving. Three of the fifteen flasks were not autoclaved, three were autoclaved 45 minutes, three 1 hour and 45 minutes, three 11 hours, and three 21 hours. After removal of the last set of flasks from the autoclave the contents of all the flasks were emptied into graduate cylinders and the total contents brought up to 80 cc. The extracts were then

filtered through No. 4 Whatman filter-paper and the filtrate tested for reducing substances by Shaffer's ('14) method for the determination of sugars.

In table II the amounts of reducing substances, calculated as mgs. of copper, are given. Ten cc. of the sample were used in each case. The potassium permanganate solution used in the titration was 1/20 normal. The reduction value for the Fehling control has been subtracted.

TABLE II
AMOUNT OF REDUCING SUBSTANCES CALCULATED AS MILLIGRAMS OF COPPER
IN 10 CC. OF THE AQUEOUS EXTRACTS

Flask no.	Interval of autoclaving	Amount of reducing substances in 10 cc. of the extracts	Average
45		2.22	
46		2.22	
47	Not autoclaved	2.22	2.22
1		5.88	
2	45 minutes	6.04	
3		5.72	5.88
4		7.68	
5	1 hour, 45 minutes	7.52	
7		7.68	7.63
8		10.99	
9	11 hours	11.13	
10		11.06
11		20.98	
12	21 hours	21.08	
13		18.76	20.27

It is clearly evident that the amount of reducing substances present in the aqueous extracts gradually increases with an increase in the time of autoclaving. It is quite probable also that some of the reducing power of the aqueous extracts is due to the presence of sugars. Fromherz ('07) has shown that on treating wood with hot water under pressure considerable quantities of sugar may be formed from the lignocelluloses and other wood components. It is claimed by Schwalbe ('11) that as pure a cellulose as cotton will yield considerable quantities of sugar when subjected to the same treatment.

Reducing substances in the extracts of Douglas fir after autoclaving and subsequent treatment with 0.5 per cent sulphuric acid.—The purpose of this series is to determine whether or not previous autoclaving in any way affects the rate of acid hydrolysis of woody tissues.

Flasks containing Douglas fir sawdust and distilled water were prepared and autoclaved as before. Enough c.p. sulphuric acid was then added to each flask to make it 0.5 per cent acid, and the entire series again autoclaved for 1 hour at 15 pounds pressure and the amounts of reducing substances determined as mgs. of copper.

TABLE III
AMOUNT OF REDUCING SUBSTANCES CALCULATED AS MILLIGRAMS OF COPPER
IN 10 CC. OF THE EXTRACTS

Flask no.	Interval of autoclaving with dist. H ₂ O	Amount of reducing substance in 10 cc. of the extracts	Average
40		55.97	
41	Not autoclaved	55.97	55.97
14		72.22	
15	45 minutes	57.08	64.65
17		58.83	
19	11 hours	58.83	58.83
23		61.05	
24	21 hours	63.60	62.32

The results here show but a slight increase, if any, in the amounts of reducing substances present in the wood extracts in the flasks regardless of the increased time of autoclaving with distilled water. This may indicate either that prolonged previous treatment with distilled water did not cause any changes in the wood which would make it more subject to acid hydrolysis or that 0.5 per cent sulphuric acid is of sufficient concentration to hydrolyze the wood in any condition.

Reducing substances in the extracts of Douglas fir after autoclaving and subsequent treatment with 1.0 per cent sodium hydroxide.—Flasks containing 2.000 gms. of Douglas fir sawdust were prepared in exactly the same manner as in the two

previous series. After autoclaving the flasks and contents for varying intervals with distilled water enough c. p. sodium hydroxide was added to each flask to make the contents 1.0 per cent alkaline, and the entire series was again autoclaved for one hour at fifteen pounds and the amount of reducing substances determined as mgs. of copper.

TABLE IV
AMOUNTS OF REDUCING SUBSTANCES CALCULATED AS MILLIGRAMS OF COPPER
IN 10 CC. OF THE EXTRACTS

Flask no.	Interval of autoclaving with dist. H_2O	Amount of reducing substances in 10 cc. of the extracts	Average
43	Not autoclaved	6.99	7.31
44		7.63	
25	45 minutes	4.08	4.24
27		4.40	
28	11 hours	5.72	6.04
29		6.36	
33	21 hours	9.54	9.46
36		9.38	

Omitting those flasks which had no previous autoclaving there is a steady increase in the amount of reducing substances as the length of previous autoclaving increases. In this series different substances from those affected by acids would, of course, be acted upon. One to two per cent solutions of alkalis, while having but little effect upon cellulose at temperatures considerably above 100° C., break down the pectic bodies and hydrolyze gums and resins which are but slightly affected with dilute acids.

Total amount of soluble substances present in the extracts.—Five cc. of the various wood extracts were placed in carefully cleaned, dry watch glasses. The liquid was evaporated in a hot-air oven at a temperature of 90° C., the heating being continued until the weight of the residue remained constant. The following table shows the total dry weight in 5 cc. of the ex-

tracts when the sawdust was autoclaved with distilled water for the time indicated:

TABLE V
TOTAL DRY WEIGHT OF THE SOLUBLE SUBSTANCES IN THE AQUEOUS EXTRACTS

Flask no.	Interval of autoclaving	Dry wt. of residue (gms.)	Average (gms.)
45	Not autoclaved	0.0045	0.0046
46		0.0045	
47		0.0047	
1	45 minutes	0.0078	0.0076
2		0.0072	
3		0.0077	
4	1 hour, 45 minutes	0.0076	0.0075
5		0.0075	
7		0.0075	
8	11 hours	0.0114	0.0113
9		0.0113	
10		0.0112	
11	21 hours	0.0149	0.0143
12		0.0137	
13		0.0143	

A considerable increase in the total soluble substances is evident. This is no doubt due in part to the dissolving out of initially soluble substances, but that it may also be partly due to the conversion of some of the wood substances is indi-

TABLE VI
TOTAL DRY WEIGHT OF THE SOLUBLE SUBSTANCES IN THE ACID EXTRACTS

Flask no.	Interval of autoclaving with dist. H ₂ O	Subsequent autoclaving after addition of acid	Dry wt. of residue (gms.)	Average (gms.)
39	Not autoclaved	1 hour	0.0169	0.0180
40			0.0173	
41			0.0199	
14	45 minutes	1 hour	0.0073	0.0066
15			0.0045	
16			0.0079	
17	11 hours	1 hour	0.0088	0.0081
19			0.0080	
21			0.0075	
22	21 hours	1 hour	0.0177	0.0185
23			0.0193	
24			

cated by the table showing a similar increase in the amounts of reducing substances present.

The same procedure was followed with the extracts containing 0.5 per cent sulphuric acid. Table VI shows the dry weight of the total soluble substances after the weight of sulphuric acid had been deducted in each case. Each individual watch glass was weighed immediately after taking out of the oven, to avoid the error which might be caused by the acid taking up atmospheric moisture.

Table VII shows the total dry weight of the soluble substances present in the alkaline extracts after the amount of sodium hydroxide in the residue had been deducted in each case.

TABLE VII
TOTAL DRY WEIGHT OF THE SOLUBLE SUBSTANCES IN THE ALKALINE EXTRACTS

Flask no.	Interval of autoclaving with dist. H ₂ O	Subsequent autoclaving after addition of alkali	Dry wt. of residue (gms.)	Average (gms.)
42			0.0366	
43			0.0312	
44			0.0333	0.0337
25			0.0384	
26	45 minutes	1 hour	0.0385	
27			0.0377	0.0382
28*			0.0100	
29*	11 hours	1 hour	0.0029	
31			0.0064
32			0.0383	
33	21 hours	1 hour	0.0415	
36			0.0413	0.0404

* Portion of extract lost in oven.

Here again a slight increase is noted as the interval of autoclaving with distilled water increased.

Relative amounts of tannins present in the aqueous extracts.—It could legitimately be contended that the increase in the amounts of reducing substances present in the extracts which reduce Fehling's solution might merely be due to prolonged boiling extracting greater amounts of tannin. With this in mind the aqueous extracts were treated with a solution of ferric chloride and the intensity of the color taken as a crite-

riion of the relative amounts of tannins present. Five cc. of the various aqueous extracts were placed in clean test-tubes of 18 mm. diameter, four drops of a 2.0 per cent aqueous solution of ferric chloride added to each, and the color determined by comparing them with Ridgway's ('12) plates.

TABLE VIII
RELATIVE AMOUNTS OF TANNINS PRESENT IN THE AQUEOUS EXTRACTS

Flask no.	Interval of autoclaving	Color	Order of intensity
45, 46, 47.....	Not autoclaved	Wood-brown	3
1, 2, 3.....	45 minutes	Yew-green	1
4, 5, 7.....	1 hour, 45 minutes	Krönberg's green	2
8, 9, 10.....	11 hours	Wood-brown	3
11, 12, 13.....	21 hours	Wood-brown	3
Control (distilled water)	Yellow tint

This would seem to indicate that the extracts from the wood autoclaved the longest intervals and the extract from the wood not autoclaved contained the least tannin, while that autoclaved for forty-five minutes contained the most. Comparing these results with the table showing the amounts of reducing substances in the various aqueous extracts it is found that they in no way coincide.

The presence of lignin in the aqueous extracts.—A test for the presence of lignin in the various aqueous extracts was made with phloroglucin and hydrochloric acid. In no case was more than a faint pink color evident. When, however, a few cc. of ether were added, the mixture well shaken, the ether separated and evaporated, the residue gave a decided red color with phloroglucin and hydrochloric acid. All of the extracts gave a positive test when treated in this manner.

The presence of coniferin in the aqueous extracts.—When an aqueous solution of phenol and hydrochloric acid was added to the extracts in no case did a color reaction take place.

Hydrogen ion concentration of the aqueous extracts.—The hydrogen ion concentration of the extracts was determined according to the method suggested by Sörensen ('09) as modified by Henderson ('09) and previously employed by Duggar

in this laboratory. The following table gives the results of these determinations:

TABLE IX
HYDROGEN ION CONCENTRATION OF THE AQUEOUS EXTRACTS

Flask no.	Interval of autoclaving	Hydrogen ion concentration
45, 46, 47.....	Not autoclaved	10^{-7}
1, 2, 3.....	45 minutes	10^{-6}
4, 5, 7.....	1 hour, 45 minutes	10^{-6}
8, 9, 10.....	11 hours	10^{-5}
11, 12, 13.....	21 hours	10^{-4}

The extract from the unautoclaved Douglas fir sawdust is practically neutral. With the autoclaved sawdust there is a decided increase in the hydrogen ion concentration in the aqueous extracts as the interval of autoclaving increases.

Acidity of the aqueous extracts.—The extracts were also titrated against N/20 sodium hydroxide with the following results. The acidity is expressed according to Fuller's scale, each being the average of three titrations.

TABLE X
DEGREE OF ACIDITY OF THE AQUEOUS EXTRACTS

Flask no.	Interval of autoclaving	Acidity
46, 47.....	Not autoclaved	1.0
2, 3.....	45 minutes	1.0
5, 7.....	1 hour, 45 minutes	2.0
8, 9.....	11 hours	3.0
12, 13.....	21 hours	4.0

These results correspond with the hydrogen ion determinations. A gradual increase in acidity results from increased length of autoclaving.

Effect of autoclaving on the rate of decay induced by fungi.—In order to determine whether or not autoclaving in any way affected the rate of decay induced by fungi approximately equal amounts of western hemlock sawdust were placed in 125-cc. wide-necked bottles. The bottles and contents were placed in the hot-air oven and dried to constant weight at a temperature of 90° C. and then weighed. Approximately equal

amounts of distilled water were added to each bottle and they were then autoclaved for periods from 45 minutes to 20 hours.

The bottles in which the contents were not autoclaved were treated as follows: After drying to constant weight in the hot-air oven they were plugged with previously sterilized plugs. Weighing was done with the plugs removed, every precaution being taken to avoid contamination. Sterile distilled water was then added and they were ready for inoculation. Portions of the contents of three test bottles treated in this manner were poured on sterile agar plates and in each case they were found to be sterile.

All bottles were inoculated with *Fomes pinicola* and the rate of decay determined by the loss in weight of the sawdust at the end of four months. The inoculations were made on April 16, 1917. On August 18, 1917, the plugs were removed and the contents again dried to constant weight and the loss in weight determined. Each series contained eight cultures.

The details of this work are discussed under the relation of bacteria to the rate of decay induced by fungi.

Table XI clearly shows that autoclaving western hemlock has a marked effect on the rate of decay induced by *Fomes pinicola*. It is quite probable that for different fungi the effect might be quite different. In this case a gradual increase in the rate of decay is noted with an increase in time of autoclaving up to ten hours. After twenty hours of autoclaving the fungus did not attack the wood even though each bottle, in this case, was inoculated the second time. There was no growth of the fungus from the immediate point of inoculation, and when, at the time of the second inoculation, 30 days after the first, the small mass of fungus and agar used for the first inoculation was removed from the bottles and transferred to agar slants the fungus was found to be dead. At some point between ten and twenty hours' autoclaving a decided change in the sawdust must have taken place which made it toxic to the fungus. Just what this change was is not known. Suffice it here to say that when rate of decay induced by fungi is studied the time of autoclaving of the wood must also receive due consideration.

TABLE XI

THE EFFECT OF AUTOCLAVING WESTERN HEMLOCK ON THE RATE OF DECAY
INDUCED BY *FOMES PINICOLA*

I	II	III	IV	V	VI	VII	VIII
Culture bottle	Weight of saw-dust before decay (gms.)	Weight of saw-dust after decay (gms.)	Loss in weight (gms.)	Loss (per cent)	Average loss (per cent)	Time of autoclaving	Remarks
434	7.34	7.33	0.01	0.13		Not autoclaved	Only slight growth of fungus near point of inoculation.
655	7.16	7.16	0.00	0.00			
242	7.58	7.58	0.00	0.00			
160	7.25	7.25	0.00	0.00			
106	6.96	6.96	0.00	0.00			
582	7.21	7.21	0.00	0.00	0.01		
16	7.19	7.18	0.01	0.14		45 minutes	Light growth on surface.
558	7.90	7.89	0.01	0.13			
741	7.71	7.71	0.00	0.00			
595	8.94	8.92	0.02	0.22			
244	7.46	7.44	0.02	0.27	0.15		
724	8.09	8.07	0.02	0.23		5 hours	Light growth throughout entire mass of sawdust.
598	7.00	6.96	0.04	0.57			
99	6.86	6.82	0.04	0.58			
209	7.49	7.42	0.07	0.93			
120	7.47	7.42	0.05	0.67			
762	7.67	7.62	0.05	0.65			
652	6.88	6.87	0.01	0.14	0.54		
739	6.95	6.35	0.60	8.63		10 hours	Heavy growth throughout entire mass of sawdust.
744	6.64	6.48	0.16	2.41			
647	7.50	7.32	0.18	2.40			
261	6.81	6.74	0.07	1.03			
602	7.59	7.57	0.02	0.26			
477	7.30	7.26	0.04	0.55			
98	7.40	7.34	0.06	0.81	2.30		
683	7.13	7.13	0.00	0.00		20 hours	All bottles inoculated twice. In neither case did infection take place.
408	7.31	7.31	0.00	0.00			
95	7.17	7.17	0.00	0.00			
579	7.23	7.22	0.01	0.14			
644	7.54	7.54	0.00	0.00			
610	7.31	7.31	0.00	0.00			
154	7.53	7.53	0.00	0.00	0.01		
1051	9.37	9.37	0.00	0.00		Control	Error due to weighing ± 0.02 per cent.
1052	8.53	8.53	0.00	0.00			
1053	9.11	9.11	0.00	0.00			
1054	8.60	8.60	0.00	0.00			
1055	7.32	7.32	0.00	0.00			
1056	7.47	7.46	0.01	0.13			
1057	9.23	9.23	0.00	0.00			
1058	6.04	6.04	0.00	0.00			
1059	8.53	8.53	0.00	0.00			
1060	9.16	9.15	0.01	0.11	0.02		

BACTERIA IN RELATION TO CELLULOSE FERMENTATION AS
INDICATED BY THE DECAY OF WOOD

Isolation of bacteria present on decaying wood under natural conditions and the determination of their cellulose-dissolving properties.—It has been satisfactorily demonstrated by numerous investigators that certain bacteria have the power to dissolve cellulose under both aërobic and anaërobic conditions. If these forms play any part in the decay of wood under natural conditions they should be found present in connection with the fungi or alone on decaying wood. To determine this the bacteria from numerous samples of decaying wood were isolated and their cellulose-dissolving properties investigated.

Method of isolation.—Small samples of decaying wood were placed in test-tubes containing about 10 cc. of sterile distilled water and allowed to soak from five to ten minutes with frequent vigorous shakings of the tube to facilitate removing any bacteria which might adhere to the surface of the samples. Transfers were made from the water blanks to tubes of melted media and plated in the usual manner.

The following kinds of media were employed: hard potato agar, cotton cellulose agar, filter-paper cellulose agar, oak cellulose agar, ash cellulose agar, Douglas fir cellulose agar, and western hemlock cellulose agar.

The hard potato agar had the following composition:

Extract from	200 gms. potato
Glucose	20 gms.
Agar	20 gms.
Distilled water to make	1000 cc.

The filter-paper cellulose was prepared in the manner described by McBeth and Scales ('13) and later by Cooley ('14). Fifteen gms. of filter-paper were dissolved in Schweitzer's solution and precipitated with hydrochloric acid. The mixture was diluted to ten liters and the cellulose allowed to settle. After settling, the supernatant liquid was poured off and the cellulose thoroughly washed with hydrochloric acid to get rid of all traces of copper. It was then thoroughly washed with

distilled water to remove all traces of chlorine. The water was filtered off with a Buchner's funnel and the cellulose washed repeatedly. After washing, it was transferred to a liter flask containing 500 cc. of distilled water, and sterilized.

Cotton cellulose was prepared in the same manner as the above.

The wood cellulose was prepared by treating a quantity of fine shavings of the wood in question with a solution composed of 30 gms. of potassium chlorate dissolved in 520 cc. of nitric acid (sp. gr. 1.1). The shavings were added slowly to the solution while the flask was immersed in ice water to prevent excessive heating. After the addition of the shavings the mixture was kept cool for four to five weeks when the shavings were well washed and then dissolved in Schweitzer's solution in the same manner as was the filter-paper and the cotton.

Cellulose agar was prepared by adding about 1 per cent (by weight) of the precipitated celluloses to a mineral nutrient solution having the following composition:

Monopotassium phosphate	1 gm.
Magnesium sulphate	1 gm.
Sodium chloride	1 gm.
Ammonium sulphate	1 gm.
Calcium carbonate	2 gms.
Distilled water	1000 cc.

Twenty gms. of agar were added to a mixture containing 500 cc. of the above solution and 500 cc. of the cellulose suspension.

It is evident that the cellulose present in media made up in this manner is the only source of carbon. Growth of an organism, therefore, on the medium may be taken as an indication of the power to utilize cellulose. Table XII indicates the results of the attempts to isolate cellulose-dissolving organisms from decaying wood. The cellulose agar plates were kept under observation from two to three weeks at room temperature.

In no case was a bacterial form found on any of the cellulose agars. The fact that none developed, however, does not preclude the possibility of their presence on the decaying wood. It is a known fact that some organisms require the presence

TABLE XII
ISOLATION OF CELLULOSE-DISSOLVING BACTERIA FROM DECAYING WOOD

Sample no.	Place of collection	Date of collection	Kind of wood	No. of bacterial colonies on Thaxter's agar	Number of colonies on cellulose agar					
					Filter paper	Cotton	Ash	Oak	Douglas fir	Western hemlock
1	St. Louis	10/ 3/16	Willow	0	0	0	0	0	0	0
2		10/ 3/16	Willow	8	0	0	0	0	0	0
3		11/24/16	Locust	4	0	0	0	0	0	0
4		11/24/16	Locust	6	0	0	0	0	0	0
5		11/24/16	Ash	3	0	0	0	0	0	0
6		11/24/16	Osage orange	9	0	0	0	0	0	0
7		11/24/16	Juniper	2	0	0	0	0	0	0
8		11/24/16	Oak	3	0	0	0	0	0	0
9		11/24/16	Oak	2	0	0	0	0	0	0
10		11/27/16	Palm stem	5	0	0	0	0	0	0
11	Seattle	10/10/16	Douglas fir	2	0	0	0	0	0	0
12		10/10/16	Douglas fir	0	0	0	0	0	0	0
13		10/10/16	Douglas fir	0	0	0	0	0	0	0
14		10/10/16	Douglas fir	0	0	0	0	0	0	0
15		10/10/16	Douglas fir	3	0	0	0	0	0	0
16		10/10/16	Douglas fir	7	0	0	0	0	0	0
17		10/10/16	Douglas fir	4	0	0	0	0	0	0
18		10/10/16	Douglas fir	6	0	0	0	0	0	0
19		10/10/16	Western hemlock	7	0	0	0	0	0	0
20		10/10/16	Western hemlock	0	0	0	0	0	0	0
21		10/10/16	Western hemlock	2	0	0	0	0	0	0
22		10/10/16	Western hemlock	3	0	0	0	0	0	0
23		10/10/16	Western hemlock	0	0	0	0	0	0	0
24		10/10/16	Western hemlock	1	0	0	0	0	0	0
25		10/10/16	Western hemlock	3	0	0	0	0	0	0
26		10/10/16	Western hemlock	3	0	0	0	0	0	0
27		10/10/16	Western hemlock	2	0	0	0	0	0	0
28		10/10/16	Western hemlock	1	0	0	0	0	0	0
29		10/10/16	Western hemlock	0	0	0	0	0	0	0
31		10/10/16	Western hemlock	4	0	0	0	0	0	0
32	St. Louis	10/10/16	Ash	1	0	0	0	0	0	0
33		10/10/16	Ash	4	0	0	0	0	0	0
34		10/10/16	Maple	3	0	0	0	0	0	0
35		10/10/16	Maple	5	0	0	0	0	0	0

of small quantities of other carbohydrates in their early stages of development before cellulose can be utilized. In many of the plates species of *Penicillium* developed which dissolved the cellulose to a considerable extent. This could be determined by the clearing up of the agar near the colonies. That some species of *Penicillium* have this power has been shown by Ward ('98) and McBeth and Scales ('13).

RELATION OF BACTERIA TO THE RATE OF DECAY INDUCED BY
FUNGI UNDER LABORATORY CONDITIONS

It is possible that even though the cellulose-dissolving bacteria play no part in the decay of wood under natural conditions, the ordinary air, soil, and water forms, so universally found in connection with decaying wood, may have an important indirect influence on the rate of decay caused by fungi. This may come about by these organisms changing the reaction of the substratum in such a manner that it may be more or less favorable to the optimum development of the fungus; or it is also quite possible that in their metabolism they may utilize some of the hydrolytic products produced by the fungus, an accumulation of which might be partially toxic to the fungus. In order to determine whether or not this is true, pure cultures of bacteria were added to pure cultures of different fungi and their effect, if any, on the rate of decay noted.

Methods and materials.—Approximately equal amounts of sawdust were placed in clean weighed 125-cc. wide-necked bottles. The bottles and contents were placed in a hot-air oven and dried to constant weight at a temperature of 90° C. and weighed again. Approximately equal amounts of water were added to each flask and they were then plugged and sterilized at fifteen pounds for twenty minutes. Every effort was made to have conditions as nearly identical as possible in each bottle. Each was inoculated with a wood-destroying fungus, and after the wood was well infected bacteria were added and the rate of decay determined by the loss in weight of the culture bottles after six months.

The experiments were conducted with four species of commercial woods, Douglas fir (*Pseudotsuga taxifolia*), western hemlock (*Tsuga heterophylla*), white ash (*Fraxinus sp.*), and red oak (*Quercus sp.*). Heart-wood was used in each instance. The Douglas fir and western hemlock wood were obtained from freshly sawed logs. The white ash and red oak were purchased from lumber dealers and were in all probability air-seasoned. The sawdust was prepared as previously discussed.

CULTURE RELATIONS: FUNGI

The three fungi used were selected because of their vigorous growth in cultures, their importance as wood-destroying forms, and the fact that they are found on both coniferous and deciduous hosts. *Fomes pinicola* Fr. is particularly destructive on a great variety of conifers. It has also been reported by Weir ('14) on *Populus trichocarpa*, *P. tremuloides*, *Betula occidentalis*, *Fagus sylvatica*, *Quercus pendulata*, and other deciduous hosts. *Polystictus versicolor* (L.) Fr. has a wide range of deciduous hosts and has also been reported by Weir ('14) on *Larix occidentalis*, *Pinus monticola*, and *Pseudotsuga taxifolia*. *Lenzites saepiaria* Fr. has been previously used in this laboratory by Zeller ('16) with excellent results. It is more important as an organism causing decay of coniferous woods but has also been reported on *Populus alba*, *P. tremuloides*, *Betula occidentalis*, *Acer glabrum*, and other deciduous hosts by Weir ('14).

Cultures of *Fomes pinicola* were made from young sporophores by the tissue method first reported by Duggar ('05) and later used by Zeller ('16) in obtaining cultures of *Lenzites saepiaria*. Young sporophores, from four to six inches in diameter, were washed with tap water followed by a thorough washing with sterile distilled water, and then dried with sterile tissue towelling. An incision from one-half to one centimeter deep was made across both surfaces of the sporophore with a sterile scalpel and the sporophore then broken open. This eliminated the possibility of contamination of the deeper tissue by the carrying down by the scalpel of bacteria and spores from the surface of the sporophore and also left a jagged surface on the broken deeper tissues, portions of which were easily torn off by means of a sterile forceps and quickly transferred to Thaxter's hard agar slants. The fungus grew exceptionally well on this media. Twenty-four tissue transfers were made, of which twenty-three grew, none being contaminated.

Cultures of *Lenzites* and *Polystictus* were made by the spore method. This method has been extensively used by Falck ('02), Lyman ('07), Münch ('09), and Zeller ('16). The tech-

nique involved in making spore cultures of the two forms is similar. The sporophores were thoroughly rinsed with sterile distilled water to remove as many bacteria and foreign spores of fungi as possible. Since gentle agitation facilitates this, it was found convenient to place the sporophores in sterile wide-necked flasks partially filled with sterile distilled water. After a gentle shaking the sporophores were removed with a pair of sterile forceps, dried with sterile tissue towelling and placed in other similar flasks. By repeating this process three times the surface of the sporophore is found to be comparatively free from bacteria and the spores of foreign fungi. Following this preliminary washing the sporophores were allowed to soak from one to two hours in sterile distilled water, then thoroughly rinsed and dried as before, and finally placed hymenium downward in large sterile Petri dishes. By means of short sterile glass rods the sporophore was kept from resting on the bottom of the Petri dish and thus the possibility of contamination of the Petri dish by contact with the sporophore was avoided. In from twelve hours to two days the spores were discharged to such an extent that they were plainly visible on the bottom of the Petri dish. By means of a platinum loop they were removed to a sterile water blank and by the same method were transferred from the water blank to tubes of molten agar and then plated in the usual manner. Transfers were made from characteristic colonies from the plates to agar slants.

A much easier and quicker method, though less certain to be a pure culture, is to place the washed sporophores in Petri dishes containing a small amount of sterile agar. The sporophores are not allowed to rest directly on the agar but are kept from it by means of short sterile glass rods lying across the agar. The spores thus fall directly on the nutrient agar and after germination transfers can be made directly to agar slants. Cultures free from contamination were obtained by this method but were not used on account of the decided advantages of the other method.

In order to avoid growing the fungi on artificial media for any length of time before using them in the decay experiments,

they were transferred to sterile Douglas fir and hemlock sawdust in Erlenmeyer flasks for keeping. All three forms grew comparatively well on both kinds of sawdust.

Optimum degree of acidity of media for fungi used.—In connection with this work it was necessary to determine whether or not slight changes in the reaction of the medium had any influence on the rate of growth of the fungi. Media of different degrees of acidity were therefore made up. Three liters of medium having the following composition per liter were prepared:

Extract from 450 gms. carrot	
Glucose	10 gms.
Agar	25 gms.
Distilled water to make..	1000 cc.

Two-hundred-cc. amounts of this medium were placed in 500-cc. Erlenmeyer flasks and autoclaved for twenty minutes at fifteen pounds. This medium titrated +13.5 Fuller's scale after autoclaving. After adding varying amounts of sterilized hydrochloric acid or sodium hydroxide to the flasks samples were again titrated while the agar was still in a liquid condition. Twenty-cc. amounts were placed in each of six Petri dishes of approximately nine centimeters diameter. Before inoculating these plates with the fungi they were allowed to stand for three days at room temperature in order to determine whether or not they were sterile. For each fungus two plates were used for each concentration. The colonies were measured in two directions at right angles to each other and the average taken.

It was endeavored to inoculate all plates in a practically identical manner, and to do this, plate cultures of the fungus in question were cut by means of a sterile scalpel into squares measuring approximately three-quarters of a centimeter on each side. A single square was placed in the center of each plate and after six and again after eight days' incubation at room temperature the size of the colonies was measured. The results are tabulated in table XIII:

TABLE XIII

RATE OF GROWTH OF *FOMES PINICOLA*, *POLYSTICTUS VERSICOLOR*, AND *LENZITES SAEPIARIA* ON MEDIA OF INCREASING ACIDITY

Reaction of media, Fuller's scale	Diameter of colonies in centimeters					
	<i>Fomes pinicola</i>		<i>Polystictus versicolor</i>		<i>Lenzites saepiaria</i>	
	6 days	8 days	6 days	8 days	6 days	8 days
+ 2.5	No growth	1.8	No growth	1.6	No growth	2.3
+ 4.5	1.8	3.0	4.9	7.0	2.3	3.7
+ 6.0	1.9	3.1	5.4	7.6	2.4	3.9
+ 7.75	2.0	3.1	6.3	8.3	2.4	3.9
+ 9.75	1.9	3.1	6.6	9.3	3.2	4.1
+13.5	2.1	3.3	5.7	7.4	2.4	4.0
+14.0	1.9	3.1	5.8	7.4	2.5	4.0
+16.25	1.8	2.9	5.3	6.9	2.6	3.9
+18.5	1.7	2.9	5.1	6.4	2.7	4.1
+21.25	1.8	3.0	4.7	6.0	2.5	3.9
+22.25	1.6	3.0	4.4	5.7	2.3	3.3
+24.5	1.7	3.0	4.3	4.6	2.1	2.8

There is but little difference in the rate of growth of *Fomes pinicola* on media ranging from + 5 to + 24.5 acid, Fuller's scale. On a medium of + 2.5 there is a decided retardation in the rate of growth. *Polystictus versicolor* shows a marked response to the reaction of the medium. A maximum is attained when the medium titrates + 9.75, Fuller's scale, with a steady decrease in the rate of growth as the acidity increases. The effect of the reaction of the medium on the rate of growth of *Lenzites saepiaria* is quite similar to that on *Fomes pinicola* except that there is a marked retardation on the media of the higher concentrations.

It is appreciated that by titrating samples of acidified agar in this manner the actual acidity of the medium is not definitely determined, due to the buffer effect of the agar on the hydrogen ions; yet the results would indicate that slight variations in the reaction of the substratum would have little or no effect on the rate of development of *Fomes pinicola* or *Lenzites saepiaria* but that they might have in the case of *Polystictus versicolor*.

CULTURE RELATIONS: BACTERIA

The bacteria used were chosen, with one exception, with reference to their acid or alkaline reactions on culture media. The presence of a nitrogen-fixing soil form was also tried. The

nomenclature used is that of Migula ('00). The following organisms were employed:

(1) Alkaline on litmus milk.

Bacillus vulgaris (Flügge) Mig.

Bacterium mycoides (Scholl) Mig.

(2) Acid on litmus milk.

Bacillus vulgaris (Hauser) Mig.

Bacillus coli (Escherich) Mig.

Bacillus prodigiosus (Ehrenberg) Flügge.

(3) Nitrogen-fixing form.

Azotobacter chroococcum Beijerinck.

All forms were plated in the usual manner, to make certain that pure cultures were being considered, and then transferred to standard beef broth.

Method of inoculation of culture bottles.—Transfers from the sawdust cultures of the fungi were made to sterile agar plates. When the entire surface of the plate was overgrown with the mycelium it was cut into small squares of approximately one square centimeter size by means of a sterile scalpel. A single square was added to each culture bottle. In most cases the mycelium could be seen growing through the sawdust about one week after inoculation.

When the fungus mycelium had become well established in the sawdust the culture bottles were inoculated a second time either with another fungus or with bacteria. Previous to this second inoculation all culture bottles were carefully inspected and those in which the infection of the sawdust did not occur were thrown out. The second fungus inoculation was made in a similar manner to the first. The bacteria were added by transferring a considerable number of the organisms from hard agar slants to 250-cc. Erlenmeyer flasks containing distilled water. Where more than one bacterium was added to the culture bottles a mixture of the organisms was made in the same way. Approximately one cc. of bacterium suspension was added to each culture bottle. After all of the culture bottles were inoculated with the bacterial suspension transfers were made to agar slants to make certain that the action of the

distilled water had not killed the organisms. In the case of the controls and bottles which contained the fungi alone a similar amount of sterile distilled water was added so that the moisture conditions in all the culture bottles would be the same.

After all of the culture bottles were inoculated with the desired organisms the cotton plug of each was covered with two thicknesses of paraffined tissue paper to avoid excessive evaporation from the flasks, the paper being secured by means of a rubber band. This did not make an air-tight covering, but in most cases the cultures remained sufficiently moist without watering throughout the period of incubation. In the few cases in which additional watering was necessary effort was made to keep the moisture conditions similar to those of the other culture bottles.

The cultures were then removed to a very humid rotting-pit with a temperature varying from 22° C. in summer to 35° C. in winter. The period of incubation in all cases was six months.

At the end of the period of incubation small particles of sawdust, such as would adhere to a moist sterile platinum needle were transferred to sterile potato agar slants in order to determine whether or not the bacteria were still alive. The plugs were then removed and the culture bottles again dried to constant weight in the hot-air oven at a temperature of 90° C., weighed, and the loss in weight determined.

In all cases the rate of decay is based on the loss in weight of the culture bottles during the period of incubation. Each set originally contained ten cultures but some were thrown out, due to infection of the fungus not taking. The average loss in each set was determined and taken as the basis of comparison between the various sets.

DESCRIPTION OF CULTURE SERIES

Four series of cultures were prepared: series A, of red oak; series B, of white ash; series C, of western hemlock; and series D, of Douglas fir.

Culture bottles from which transfers were made to determine the presence or absence of bacteria at the end of the

period of incubation are indicated by the symbols designated in the footnotes in the following tables:

TABLE XIV (Series A)
THE EFFECT OF BACTERIA ON THE RATE OF DECAY INDUCED BY *FOMES PINICOLA*,
POLYSTICTUS VERSICOLOR, AND *LENZITES SAEPIARIA* ON RED OAK

I	II	III	IV	V	I	II	III	IV	V
Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)	Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)
Control, error due to weighing $\pm 0.02\%$									
71	8.37	8.36	0.01	0.12	21	9.52	9.50	0.02	0.21
72	7.98	7.98	0.00	0.00	22	9.79	9.75	0.04	0.40
73	7.41	7.41	0.00	0.00	23
74	9.00	9.00	0.00	0.00	24	10.05	10.04	0.01	0.10
75	8.96	8.96	0.00	0.00	25	9.53	9.49	0.04	0.60
76	9.51	9.50	0.01	0.10	26	10.30	10.25	0.05	0.48
77	7.39	7.39	0.00	0.00	27	9.69	9.67	0.02	0.21
78	7.56	7.56	0.00	0.00	28	9.32	9.30	0.02	0.21
79	8.09	8.09	0.00	0.00	29	10.15	10.14	0.01	0.10
80	9.16	9.16	0.00	0.00	30
Average	0.02		Average	0.29	
<i>Fomes pinicola</i> 2/16/17									
1	9.44	9.41	0.03	0.32	31	9.53	9.32	0.21	2.22
2	9.47	9.47	0.00	0.00	32	8.72	8.61	0.11	1.26
3	9.45	9.45	0.00	0.00	33	9.47	9.27	0.20	2.11
4	8.71	8.70	0.01	0.11	34	10.26	10.20	0.06	0.58
5	8.87	8.84	0.03	0.38	35	9.02	8.90	0.12	1.33
6	8.83	8.83	0.00	0.00	36	9.36	9.20	0.16	1.70
7	9.51	9.48	0.03	0.31	37	9.41	9.35	0.06	0.63
8	9.03	9.03	0.00	0.00	38	9.96	9.91	0.05	0.50
9	9.34	9.33	0.01	0.11	39	9.71	9.53	0.18	1.85
10	9.00	9.00	0.00	0.00	40	9.55	9.52	0.03	0.31
Average	0.10		Average	1.24	
<i>F. pinicola</i> 2/16/17, <i>P. versicolor</i> & <i>saepiaria</i> 3/30/17									
11	8.75	8.72	0.03	0.34	41	9.23	9.18	0.05	0.54
12	9.22	9.20	0.02	0.21	42	9.35	8.95	0.40	4.27
13	9.42	9.39	0.03	0.32	43	9.94	9.86	0.08	0.80
14	9.11	9.09	0.02	0.22	44	8.70	8.43	0.27	3.08
15	45	9.45	9.26	0.19	2.01
16	46	8.97	8.91	0.06	0.67
17	9.89	9.78	0.11	1.11	47*	9.12	8.99	0.13	1.42
18	9.26	9.26	0.00	0.00	48	9.42	9.20	0.22	2.46
19	9.12	9.07	0.05	0.55	49*	9.14	9.04	0.10	1.09
20	9.95	9.92	0.03	0.30	50*	9.33	9.19	0.14	1.60
Average	0.36		Average	1.79	

* No bacteria present at end of incubation.

TABLE XIV (Continued)

I	II	III	IV	V	I	II	III	IV	V
<i>F. pinicola</i> 2/16/17, <i>Bact. mycoides</i> 3/31/17					<i>L. saepiaria</i> 2/15/17, <i>Bact. mycoides</i> 3/31/17				
51	9.47	9.16	0.31	3.37	102*	8.48	8.43	0.05	0.59
52	9.36	9.15	0.21	2.23	103	8.75	8.72	0.03	0.34
53	10.22	10.04	0.18	1.86	104	8.92	8.92	0.00	0.00
54	10.15	10.00	0.15	1.08	105	8.78	8.67	0.11	1.25
55	9.99	9.93	0.06	0.60	107	8.36	8.34	0.02	0.23
56*	9.25	9.06	0.19	2.06	108*	8.64	8.64	0.00	0.00
57	9.58	9.38	0.20	2.09	109	8.91	8.79	0.12	1.35
58	9.24	8.99	0.25	2.81	110*	8.26	8.20	0.06	0.72
59*	9.37	9.14	0.13	1.38
60*	9.91	9.67	0.24	2.52
Average	2.00	Average	0.56
<i>F. pinicola</i> 2/16/17, <i>B. prodigiosus</i> & <i>Bact. mycoides</i> 3/31/17					<i>L. saepiaria</i> 2/5/17, <i>B. prodigiosus</i> & <i>Bact. mycoides</i> 3/31/17				
61	9.74	9.49	0.25	2.57	111	9.22	9.22	0.00	0.00
62	9.90	9.69	0.21	2.12	113*	8.57	8.57	0.00	0.00
63*	8.98	8.75	0.23	2.56	116	9.93	9.86	0.07	0.75
64*	9.79	9.54	0.25	2.55	117*	9.73	9.61	0.12	1.23
65	9.59	9.49	0.10	1.04	118	9.49	9.48	0.01	0.11
66*	8.69	8.57	0.12	1.38	119	8.38	8.38	0.00	0.00
67	9.74	9.67	0.07	0.71	121	7.85	7.85	0.00	0.00
68	10.02	9.73	0.29	2.89	122*	8.89	8.89	0.00	0.00
69	9.01	8.82	0.19	2.12
70	11.17	10.97	0.20	1.79
Average	1.97	Average	0.25
<i>Lenzites saepiaria</i> 2/15/17					<i>L. saepiaria</i> 2/5/17, <i>B. vulgaris</i> & <i>Bact. mycoides</i> 4/2/17				
81	9.19	9.18	0.01	0.11	883*	10.94	10.90	0.04	0.36
82	8.87	8.78	0.09	1.01	885	9.66	9.55	0.11	1.14
83	8.75	8.74	0.01	0.11	887†‡	10.23	10.06	0.17	1.66
84	10.73	10.72	0.01	0.09	888†‡	9.06	9.03	0.03	0.33
85	10.27	10.27	0.00	0.00	889	8.80	8.78	0.02	0.23
86	8.99	8.98	0.01	0.11	890	8.72	8.68	0.04	0.46
87	8.42	8.42	0.00	0.00
88	8.61	8.60	0.01	0.12
89	9.32	9.30	0.02	0.22
90	9.45	9.44	0.01	0.11
Average	0.18	Average	0.69
<i>L. saepiaria</i> 2/15/17, <i>B. prodigiosus</i> 3/30/17					<i>L. saepiaria</i> 2/5/17, <i>B. vulgaris</i> & <i>B. coli</i> 4/2/17				
91	8.80	8.75	0.05	0.52	131*	10.07	10.01	0.06	0.60
92*	9.35	9.27	0.08	0.85	132	8.51	8.46	0.05	0.59
93	9.74	9.63	0.11	1.13	133*	10.39	10.36	0.03	0.30
94*	8.95	8.68	0.27	3.05	134	9.51	9.37	0.14	1.47
96	8.51	8.42	0.09	0.99	135	8.26	8.15	0.11	1.36
97	9.13	9.07	0.06	0.66	136	9.33	9.21	0.12	1.29
100*	8.40	8.35	0.05	0.59	137	10.67	10.44	0.23	2.16
.....	138	8.79	8.75	0.04	0.45
.....	140*	10.04	10.02	0.02	0.20
Average	1.11	Average	0.95

* No bacteria present after incubation.

† *Bacterium mycoides* present after incubation.‡ *Bacillus vulgaris* present after incubation.

TABLE XIV (Continued)

I	II	III	IV	V	I	II	III	IV	V
Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)	Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)
<i>L. saeparia</i> 2/5/17, <i>Azotobacter chroococcum</i> 4/2/17									
141*	9.58	9.47	0.11	1.15	172*	10.55	10.54	0.01	0.10
142	8.67	8.61	0.06	0.69	173*	9.40	9.25	0.15	1.60
143	9.82	9.73	0.09	0.92	174	10.08	9.95	0.13	1.28
144*	8.09	8.02	0.07	0.86	175	10.15	9.99	0.16	1.58
145	9.37	9.35	0.02	0.21	176	10.24	10.23	0.01	0.10
146*	8.44	8.41	0.03	0.35	177	9.18	9.14	0.04	0.44
147	8.76	8.67	0.09	1.03	178	8.07	7.98	0.09	1.11
148	10.35	10.16	0.19	1.86	179	10.11	10.11	0.00	0.00
149	8.43	8.43	0.00	0.00	180*	8.54	8.53	0.01	0.12
Average	0.78		Average	0.70	
<i>Polystictus versicolor</i> 2/4/17									
151	11.75	11.75	0.00	0.00	181	9.12	9.05	0.07	0.77
152	8.02	8.02	0.00	0.00	182*	10.34	10.31	0.03	0.28
153	11.15	11.15	0.00	0.00	183	9.88	9.88	0.00	0.00
155	9.18	9.18	0.00	0.00	184	9.27	9.14	0.13	1.40
156	10.25	10.25	0.00	0.00	185	10.11	10.09	0.02	0.20
157	7.74	7.73	0.01	0.13	186*	10.49	10.33	0.16	1.52
158	9.11	9.11	0.00	0.00	187	9.39	9.31	0.08	0.85
161	10.05	10.05	0.00	0.00	188	9.30	9.24	0.06	0.65
162	10.31	10.29	0.02	0.19	189*	9.16	9.10	0.06	0.65
Average	0.04		190	10.36	10.33	0.03	0.29
Average	0.04		Average	0.66	
<i>P. versicolor</i> 2/4/17, <i>B. prodigiosus</i> 3/30/17									
191*	8.63	8.61	0.02	0.23	201	9.28	9.17	0.11	1.19
192	9.01	8.98	0.03	0.33	202*	9.55	9.46	0.09	0.94
193	8.59	8.57	0.02	0.23	203*	9.31	9.11	0.20	2.14
194	8.86	8.79	0.07	0.81	204	9.90	9.73	0.17	1.72
195	10.95	10.81	0.14	1.28	205*	9.70	9.69	0.01	0.10
196*	8.59	8.49	0.10	1.24	206	10.00	9.94	0.06	0.60
197	8.86	8.77	0.09	1.01	207	10.43	10.25	0.18	1.72
198	9.35	9.35	0.00	0.00	208	8.93	8.81	0.12	1.34
199*	10.02	9.66	0.36	3.53	210	9.53	9.52	0.01	0.11
Average	0.61		Average	0.98	

* No bacteria present after incubation.

TABLE XIV (Continued)

I	II	III	IV	V	I	II	III	IV	V
<i>P. versicolor</i> 2/4/17, <i>Azotobacter chro-</i>					<i>P. versicolor</i> 2/4/17, <i>B. vulgatus</i> & <i>Bact.</i>				
<i>ococcum</i> 4/2/17					<i>mycoides</i> 4/2/17				
211*	9.12	9.12	0.00	0.00	891	8.26	8.26	0.00	0.00
212*	10.48	10.41	0.07	0.67	892	10.86	10.69	0.17	1.59
213	8.46	8.41	0.05	0.59	893	9.03	8.94	0.09	1.00
214*	9.78	9.77	0.01	0.11	894	8.73	8.63	0.10	1.14
215	8.57	8.43	0.14	1.63	895†‡	9.36	9.20	0.16	1.71
216	8.59	8.56	0.03	0.35	896	10.59	10.42	0.17	1.61
217	9.25	9.12	0.13	1.40	897†‡	9.43	9.40	0.03	0.32
218	9.25	9.20	0.05	0.54	898	9.27	9.13	0.14	1.51
219	9.52	9.49	0.03	0.37	899	8.92	8.88	0.04	0.45
220	9.70	9.70	0.00	0.00	900†‡	9.11	9.08	0.03	0.33
Average	0.57		Average	0.96	

TABLE XV (Series B)

THE EFFECT OF BACTERIA ON THE RATE OF DECAY INDUCED BY *FOMES PINICOLA*, *POLYSTICTUS VERSICOLOR*, AND *LENZITES SAEPIARIA* ON WHITE ASH

Control, error due to weighing $\pm 0.01\%$					<i>F. pinicola</i> 2/16/17, <i>L. saepiaria</i> 3/30/17				
1071	9.00	9.00	0.00	0.00	681	6.06	5.78	0.28	4.61
1072	6.17	6.17	0.00	0.00	682	5.83	5.57	0.26	4.46
1073	6.29	6.28	0.01	0.15	684	6.29	5.81	0.48	7.13
1074	5.80	5.80	0.00	0.00	685	5.58	5.10	0.48	8.59
1075	7.75	7.75	0.00	0.00	686	7.63	7.45	0.18	2.36
1076	9.35	9.35	0.00	0.00	687	6.35	5.97	0.38	6.00
1077	9.01	9.01	0.00	0.00	688	7.41	7.15	0.26	3.50
1078	9.87	9.87	0.00	0.00	689	5.94	5.45	0.49	8.25
1079	8.55	8.55	0.00	0.00	690	7.60	7.56	0.04	0.53
1080	10.24	10.24	0.00	0.00	Average	5.05	
Average	0.01						
<i>Fomes pinicola</i> 2/16/17					<i>F. pinicola</i> 2/16/17, <i>L. saepiaria</i> & <i>P. versicolor</i> 3/30/17				
661	6.17	5.93	0.24	3.89	691	6.76	4.78	1.98	28.29
663	6.50	5.96	0.54	6.79	692	5.53	3.75	1.78	32.15
664	5.74	5.54	0.20	3.49	693	6.52	4.40	2.12	33.52
665	6.70	6.55	0.15	2.24	694	6.31	4.64	1.67	31.41
666	6.04	5.77	0.27	4.47	696	6.07	4.10	1.97	32.46
667	7.08	7.02	0.06	0.85	698	5.67	3.70	1.97	34.75
668	6.26	5.98	0.28	4.47	700	6.61	4.76	1.85	28.40
669	6.77	6.61	0.16	2.36	Average	31.54	
670	5.89	5.57	0.32	5.45					
Average	4.89						
<i>F. pinicola</i> 2/16/17, <i>P. versicolor</i> 3/30/17					<i>F. pinicola</i> 2/16/17, <i>B. prodigiosus</i> 3/30/17				
671	6.15	5.49	0.66	10.73	701*	7.35	7.15	0.20	2.72
672	7.54	6.91	0.63	8.35	702	6.74	6.52	0.22	3.32
673	6.71	4.26	1.45	21.57	704	6.77	6.47	0.30	4.43
674	5.97	4.25	1.72	28.85	705	6.53	6.33	0.20	3.06
675	6.80	5.14	1.66	24.42	706	6.07	5.95	0.12	1.98
676	5.74	4.33	1.41	24.60	707*	7.25	7.15	0.10	1.38
677	6.15	5.97	0.18	2.93	708	6.07	5.33	0.74	12.18
678	6.79	5.51	1.28	18.88	709	6.67	6.28	0.39	5.84
679	5.92	4.87	1.05	17.75	710*	6.64	6.29	0.35	5.27
680	6.84	4.56	1.28	18.71	Average	4.46	
Average	17.68						

* No bacteria present after incubation.

† *Bacterium mycoides* present after incubation.‡ *Bacillus vulgatus* present

after incubation.

TABLE XV (Continued)

I	II	III	IV	V	I	II	III	IV	V
Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)	Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)
<i>F. pinicola</i> 2/16/17, <i>Bact. mycoides</i> 3/31/17									
711*	5.86	5.17	0.69	11.75	751	6.95	4.58	2.37	34.10
712	5.80	5.65	0.15	2.59	752*	6.63	4.12	2.51	37.88
713*	6.63	7.50	0.13	1.96	753	6.76	4.87	1.89	27.91
714	6.77	6.44	0.33	4.87	754	6.94	4.34	2.60	37.46
716	6.12	6.04	0.08	1.31	755	6.25	4.53	1.72	27.52
717	7.25	7.08	0.17	2.34	756*	6.98	4.99	1.99	24.98
718*	5.92	5.89	0.03	0.51	757*	7.93	5.12	2.81	35.45
719	6.15	5.79	0.36	5.85	759	7.41	5.83	1.58	21.30
720	7.56	6.99	0.57	7.53	760	7.82	5.00	2.82	36.01
Average	4.30		Average	31.40	
<i>F. pinicola</i> 2/16/17, <i>B. prodigiosus</i> & <i>Bact. mycoides</i> 3/31/17									
721	7.09	6.72	0.37	5.22	761	7.77	5.84	1.93	24.85
723	7.14	6.37	0.77	10.78	763	9.10	5.58	3.52	38.72
725	7.53	7.02	0.51	6.77	764	7.93	4.92	3.01	37.98
726*	7.18	6.72	0.46	5.61	765*	7.75	4.95	2.80	36.12
727*	6.95	6.39	0.56	8.06	766	8.12	5.23	2.89	35.61
728*	6.18	6.07	0.11	1.76	768*	8.37	5.82	2.55	30.50
729	6.66	6.60	0.06	0.90	769	6.64	4.65	1.99	30.04
730	6.86	6.85	0.01	0.15	770*	7.25	4.80	2.45	33.80
Average	4.91		Average	33.74	
<i>Lenzites saepiaria</i> 2/5/17									
742	5.71	3.82	1.89	33.10	771*	6.63	4.63	2.00	30.19
743	7.84	5.16	2.68	34.15	772	7.37	5.49	1.88	25.50
745	6.77	4.86	1.91	28.65	773	7.88	5.49	2.39	30.32
746	6.46	4.56	1.90	29.40	774*	7.88	5.40	2.48	31.50
748	6.54	4.57	1.97	30.15	775	6.19	4.07	2.12	34.30
750	6.31	4.09	2.22	35.01	776	6.47	4.78	1.69	26.13
.....		777	6.87	4.44	2.43	35.40
.....		780*	7.10	4.24	2.86	40.25
Average	31.74		Average	31.62	

* No bacteria present after incubation.

TABLE XV (Continued)

I	II	III	IV	V	I	II	III	IV	V
<i>L. saepiaria</i> 2/5/17, <i>B. vulgarus</i> & <i>Bact. mycoides</i> 4/2/17									
971†	12.34	7.91	4.43	35.90	821	7.41	3.64	3.77	50.75
972†	9.72	6.44	3.28	33.75	822	6.43	3.38	3.05	47.45
973	8.01	5.10	2.91	36.15	823	7.84	3.78	4.06	51.25
974	8.61	5.67	2.94	34.10	824*	6.85	4.64	2.21	32.30
975	7.64	5.01	2.63	34.45	825	7.38	3.48	3.90	52.75
976*	7.64	5.18	2.46	32.20	826*	7.46	3.71	3.75	50.20
977	8.20	5.41	2.79	34.01	827*	6.57	3.16	3.41	51.80
978	8.48	5.60	2.88	34.00	829	6.41	3.40	3.01	46.90
979	8.08	5.61	2.47	30.55	830	6.49	3.20	3.29	50.75
980	8.43	5.71	2.72	32.23
Average	33.73	Average	48.24
<i>L. saepiaria</i> 2/5/17, <i>B. vulgaris</i> & <i>B. coli</i> 4/2/17									
791	6.85	4.18	2.67	38.95	831	7.02	5.01	2.01	28.65
792	7.19	5.44	2.75	38.26	832	6.72	3.20	3.52	53.20
793*	6.69	4.21	2.48	37.08	833	7.76	4.04	3.72	48.90
794	6.52	4.04	2.48	38.00	834*	7.16	4.01	3.15	41.60
795*	7.26	4.31	2.95	40.01	835	7.53	3.91	3.62	46.75
796	6.90	4.07	1.83	26.53	836*	5.99	3.16	2.83	47.30
797	6.83	4.45	2.38	34.82	837	7.27	3.98	3.29	45.25
798*	6.83	4.33	2.50	36.60	838*	5.93	2.98	2.95	49.76
799	5.56	3.91	1.65	29.68	839	7.75	4.09	3.66	47.08
800	5.89	3.84	2.05	34.90	840	6.05	3.06	2.99	49.48
Average	35.48	Average	45.70
<i>L. saepiaria</i> 2/5/17, <i>Azotobacter chroococcum</i> 4/2/17									
801	5.68	3.25	2.43	42.80	841	7.52	3.99	3.53	47.00
802	5.87	3.19	2.68	45.60	842*	6.71	3.82	2.89	43.03
803*	6.20	3.81	2.39	38.55	843	7.81	4.10	3.71	47.50
804*	6.09	3.60	2.49	40.95	844	8.19	4.38	3.81	46.60
805*	7.41	5.31	2.10	27.32	845	7.95	3.46	4.49	56.44
806	5.90	3.70	2.20	37.30	846*	6.97	3.49	3.48	49.95
807	7.70	4.52	3.18	41.25	847	7.47	3.84	3.63	48.70
808	6.84	4.32	2.52	36.85	848†	7.60	3.60	4.00	52.70
809	5.97	3.47	2.50	41.80	849	7.41	4.07	3.34	45.01
810	6.54	3.83	2.71	41.50	850	7.85	3.78	4.07	51.80
Average	39.39	Average	48.87
<i>Polystictus versicolor</i> 2/5/17									
811	5.31	4.06	1.25	23.53	861	7.18	3.35	3.83	53.40
812	5.10	4.43	0.67	13.13	862	7.85	3.83	4.02	51.20
813	6.42	5.22	1.20	18.70	863	7.16	3.96	3.20	44.70
814	7.91	4.26	3.65	46.08	864*	6.41	3.55	2.86	44.60
815	7.42	3.99	3.43	46.23	865	8.45	4.22	4.23	50.10
816	6.89	4.99	1.90	25.60	867*	6.79	3.54	3.25	47.95
817	7.73	5.54	2.19	28.33	868*	7.52	3.78	3.74	49.70
818	6.67	5.35	1.32	19.78	869	7.58	3.82	3.76	49.60
819	7.43	4.28	3.15	42.43	870	7.02	3.63	3.39	48.30
820	7.20	5.23	1.97	27.35
Average	29.11	Average	48.84

* No bacteria present after incubation.

† *Bacterium mycoides* present after incubation.‡ *Bacillus vulgarus* present after incubation.

TABLE XV (Continued)

I Culture bottle	II Weight of sawdust before decay (gms.)	III Weight of sawdust after decay (gms.)	IV Loss in weight due to decay (gms.)	V Loss due to decay (per cent)	I Culture bottle	II Weight of sawdust before decay (gms.)	III Weight of sawdust after decay (gms.)	IV Loss in weight due to decay (gms.)	V Loss due to decay (per cent)
<i>P. versicolor</i> 2/5/17, <i>Azotobacter chro-</i> <i>ococcum</i> 4/2/17									
871	7.03	3.39	3.64	51.75	982	8.54	4.37	4.17	48.75
872	6.59	3.23	3.36	51.10	983	7.44	3.81	3.63	48.80
873	5.59	3.02	2.57	46.00	984	8.74	4.36	4.38	50.10
874	6.33	3.37	2.96	46.75	985	8.03	3.94	4.09	50.80
875*	5.70	2.74	2.96	51.90	986*	7.63	4.03	3.60	47.15
876*	5.80	3.94	1.86	32.00	987	8.28	3.81	4.47	53.90
877	6.29	3.12	3.17	50.45	988*	7.64	5.42	2.22	29.10
878*	6.59	3.13	3.46	52.50
879	7.07	3.90	3.17	44.80
880	6.50	3.33	3.17	48.75
Average	47.60		Average	46.94	

TABLE XVI (Series C)

THE EFFECT OF BACTERIA ON THE RATE OF DECAY INDUCED BY FOMES PINICOLA, POLYSTICTUS VERSICOLOR, AND LENZITES SAEPIARIA ON WESTERN HEMLOCK

Control, error due to weighing $\pm 0.02\%$					<i>F. pinicola</i> 2/16/17, <i>P. versicolor</i> 3/29/17				
1051	9.37	9.37	0.00	0.00	235	7.78	7.26	0.52	6.68
1052	8.53	8.53	0.00	0.00	236	6.10	5.95	0.15	2.46
1053	9.11	9.11	0.00	0.00	237	6.25	6.15	0.10	1.60
1054	8.60	8.60	0.00	0.00	239	7.31	7.08	0.23	3.15
1055	7.32	7.32	0.00	0.00	240	6.75	8.63	0.12	1.78
1056	7.47	7.46	0.01	0.13	241	6.69	6.58	0.11	1.64
1057	9.23	9.23	0.00	0.00	245	6.75	6.38	0.37	5.49
1058	6.04	6.04	0.00	0.00
1059	8.53	8.53	0.00	0.00
1060	9.16	9.15	0.00	0.11
Average	0.02		Average	3.26	
<i>Fomes pinicola</i> 2/16/17									
222	7.43	7.25	0.18	2.48	246	7.52	6.46	1.06	14.62
223	4.78	5.43	0.35	7.32	247	7.88	6.29	1.59	21.90
224	7.27	7.19	0.08	1.67	248	7.45	6.67	0.78	10.48
225	6.06	5.92	0.14	2.31	249	7.25	7.12	0.13	1.79
226	7.28	6.96	0.32	4.40	250	7.48	6.73	0.75	10.05
227	7.55	7.41	0.14	1.85	251	9.60	8.63	0.97	10.10
.....	252	7.48	7.30	0.18	2.42
.....	253	7.18	7.10	0.08	1.11
Average	3.34		Average	10.31	

* No bacteria present after incubation.

TABLE XVI (Continued)

I	II	III	IV	V	I	II	III	IV	V
<i>F. pinicola</i> 2/16/17, <i>P. versicolor</i> & <i>L. saepiaria</i> 3/30/17					<i>Lenzites saepiaria</i> 2/5/17				
255	7.47	6.86	0.61	8.16	301	6.96	6.33	1.63	23.40
256	6.97	6.22	0.75	10.75	302	6.25	5.58	0.67	10.73
257	8.01	7.06	0.95	11.87	303	7.22	5.81	1.41	19.51
258	7.02	6.29	0.73	10.40	304	7.94	6.06	1.88	23.70
259	7.58	6.67	0.91	12.00	305	7.52	5.93	1.59	21.12
262	8.00	7.07	0.93	11.62	306	6.66	5.53	1.13	16.95
263	7.66	6.93	0.73	9.53	307	7.17	5.56	1.61	22.41
.....	308	6.87	5.40	1.47	21.40
.....	309	7.29	5.85	1.44	19.70
.....	310	6.45	5.19	1.26	19.52
Average	10.72	Average	19.84
<i>F. pinicola</i> 2/16/17, <i>B. prodigiosus</i> 3/30/17					<i>L. saepiaria</i> 2/5/17, <i>B. prodigiosus</i> 3/30/17				
265*	6.42	6.09	0.33	5.15	311*	7.22	5.40	1.82	25.20
266	7.13	6.83	0.30	4.21	312	7.05	5.02	2.03	28.80
267	9.03	8.96	0.07	0.77	313	8.46	6.32	2.14	25.22
269	6.55	6.27	0.28	4.28	314	7.87	5.90	1.97	25.02
270*	6.84	6.75	0.09	1.32	315*	7.62	5.80	1.82	23.84
271*	9.02	8.32	0.70	7.77	316	6.42	4.86	1.56	24.30
272	7.61	7.11	0.50	6.56	317*	7.05	5.33	1.72	24.40
273	7.48	6.90	0.58	7.76	318	6.85	5.03	1.82	26.58
.....	319	7.84	6.95	0.89	11.36
.....	320	7.64	5.69	1.95	24.90
Average	4.72	Average	23.96
<i>F. pinicola</i> 2/16/17, <i>Bact. mycoides</i> 3/30/17					<i>L. saepiaria</i> 2/5/17, <i>Bact. mycoides</i> 3/31/17				
274	6.24	6.13	0.11	1.76	321	7.78	5.50	2.28	28.30
275†	7.59	7.31	0.28	3.69	322	7.53	5.55	1.98	26.31
276	7.37	7.07	0.30	4.07	323	6.94	5.38	1.56	22.52
277	6.98	6.60	0.38	5.44	324	7.32	5.41	1.91	26.10
278	7.32	6.91	0.41	5.60	325†	6.54	4.78	1.76	26.90
279	9.10	8.79	0.31	3.41	326	6.10	4.37	1.73	28.34
280†	7.48	7.10	0.38	5.09	328	6.67	5.15	1.52	22.80
281†	7.27	6.92	0.35	4.81	329†	7.36	5.45	1.91	25.92
282	8.32	7.92	0.40	4.81	330†	6.44	4.73	1.71	26.60
.....	Average	25.98
Average	4.29
<i>F. pinicola</i> 2/16/17, <i>B. prodigiosus</i> & <i>Bact. mycoides</i> 3/31/17					<i>L. saepiaria</i> 2/5/17, <i>B. prodigiosus</i> & <i>Bact. mycoides</i> 3/31/17				
283	8.00	7.90	0.10	1.25	331	6.86	5.40	1.46	21.28
284	7.53	7.03	0.50	6.64	332†	7.43	5.72	1.71	23.00
285	7.18	6.89	0.29	4.04	333	6.49	4.89	1.60	24.66
286†	6.83	6.55	0.28	4.10	334	7.34	5.52	1.82	24.78
287†	7.95	7.54	0.41	5.15	335	8.16	6.01	2.15	26.30
288	7.10	6.75	0.35	4.92	336	7.12	5.30	1.82	25.56
289	6.45	6.17	0.28	4.45	337†	7.76	5.65	2.11	27.13
290†	7.39	7.03	0.36	4.88	338	8.32	6.28	2.04	24.51
291	7.60	7.31	0.29	3.82	339	7.34	6.05	1.29	17.58
.....	340†	7.82	5.60	1.22	15.61
Average	4.36	Average	23.04

* No bacteria present after incubation.

† *Bacterium mycoides* present after incubation.

TABLE XVI (Continued)

I	II	III	IV	V	I	II	III	IV	V
Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)	Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)
<i>L. saepiaria</i> 2/5/17, <i>B. vulgaris</i> & <i>B. coli</i> 4/2/17									
351	6.97	5.11	1.86	26.70	371	7.19	7.10	0.09	1.25
352	7.31	5.39	1.92	26.28	372	6.90	6.79	0.11	1.59
353	7.66	5.69	1.97	25.80	373	7.82	7.67	0.15	1.92
354	7.19	5.23	1.96	27.28	374	7.05	7.03	0.02	0.28
355*	6.90	5.35	1.55	22.50	375	7.29	7.16	0.13	1.78
356	6.95	5.25	1.70	24.48	376	9.92	9.81	0.11	1.11
357*	7.08	5.35	1.73	24.42	377	8.53	8.40	0.13	1.52
358	7.95	5.87	2.08	26.18	379	8.62	8.54	0.08	0.93
359*	7.55	5.51	2.04	27.03	380	9.17	8.99	0.18	1.96
360	6.96	5.33	1.63	23.40
Average	25.41	Average	1.37
<i>L. saepiaria</i> 2/5/17, <i>Azotobacter chroococcum</i> 4/2/17									
361	7.54	5.62	1.92	25.48	381	8.23	7.84	0.39	4.24
362	6.36	5.49	1.87	29.45	382	7.43	7.24	0.19	2.55
363	7.56	5.53	2.03	26.86	384	6.76	6.45	0.31	4.59
364	6.52	5.83	1.69	25.91	385*	8.19	7.94	0.25	3.06
365	7.89	5.65	2.24	28.40	386	7.01	6.59	0.42	6.00
366*	7.77	5.69	2.08	26.76	387*	9.63	9.18	0.45	4.67
367	8.97	6.61	2.36	26.26	388	7.66	7.25	0.41	5.35
368	7.33	5.27	2.06	28.10	389*	8.40	7.98	0.42	5.00
369*	7.08	5.46	1.62	22.88
370*	7.23	5.26	1.97	27.26
Average	26.74	Average	4.43
<i>L. saepiaria</i> 2/5/17, <i>B. vulgatus</i> & <i>Bact. mycoides</i> 4/2/17									
911	6.82	5.19	1.63	23.92	392	8.78	7.38	0.40	4.56
912*	7.14	5.38	1.76	24.70	393†	8.29	8.05	0.24	3.18
913	9.08	7.04	2.04	22.48	394	7.22	6.87	0.35	4.85
914*	7.15	5.17	1.98	27.72	395†	6.62	6.46	0.16	2.42
915	7.10	5.38	1.72	24.25	397	7.60	7.17	0.43	5.66
916	7.87	5.84	2.03	25.85	398	6.39	6.26	0.13	2.04
917*	7.64	5.91	1.73	22.64	399	7.53	7.36	0.17	2.26
918	7.58	5.21	2.37	31.32	400†	7.39	7.22	0.17	2.30
919	7.79	5.75	2.04	26.23
920	7.86	5.78	2.08	26.45
Average	25.55	Average	3.41

* No bacteria present after incubation.

† *Bacterium mycoides* present after incubation.

TABLE XVI (Continued)

I	II	III	IV	V	I	II	III	IV	V
<i>P. versicolor</i> 2/5/17, <i>B. prodigiosus</i> & <i>Bact. mycoides</i> 3/30/17					<i>P. versicolor</i> 2/5/17, <i>Azotobacter chroococcum</i> 4/2/17				
401†	8.29	8.04	0.25	3.02	431	7.43	7.28	0.15	2.02
402	8.19	8.05	0.14	1.71	432	6.41	6.32	0.09	1.40
403	7.10	6.90	0.20	2.82	433	8.28	8.13	0.15	1.81
404	8.65	8.30	0.35	4.05	435	7.38	7.19	0.19	2.57
405	6.27	6.03	0.24	3.82	436*	7.57	7.22	0.35	4.62
406	7.13	6.97	0.16	2.24	437*	7.09	6.89	0.20	2.53
407	7.75	7.56	0.19	2.45	438	7.68	7.60	0.08	1.04
409†	7.14	6.95	0.19	2.66	439*	8.46	8.22	0.24	2.83
410†	8.86	8.68	0.18	2.03	440	8.35	8.11	0.24	2.87
Average	2.47	Average	2.41
<i>P. versicolor</i> 2/5/17, <i>B. vulgaris</i> & <i>B. coli</i> 4/2/17					<i>P. versicolor</i> 2/5/17, <i>B. vulgaris</i> & <i>Bact. mycoides</i> 4/2/17				
421	7.64	7.49	0.15	1.96	921	7.56	7.34	0.22	2.91
422*	8.09	7.81	0.28	3.46	922†	6.76	6.60	0.16	2.37
423*	9.84	7.36	0.48	4.48	923	6.79	6.66	0.13	1.92
424	8.02	7.68	0.34	4.23	924†	7.11	6.91	0.20	2.81
425	7.63	7.44	0.19	2.49	925	6.98	6.77	0.21	3.01
426*	6.47	6.36	0.11	1.70	926	7.18	7.02	0.16	2.23
427	7.30	7.15	0.15	2.06	927	7.00	6.75	0.25	3.58
428	7.45	7.25	0.20	2.68	928	6.46	6.25	0.21	3.24
429	7.33	6.97	0.36	4.90	929†	7.36	7.11	0.25	3.39
Average	3.15	Average	2.83

TABLE XVII (Series D)

THE EFFECT OF BACTERIA ON THE RATE OF DECAY INDUCED BY *FOMES PINICOLA*, *POLYSTICTUS VERSICOLOR*, AND *LENZITES SAEPIARIA* ON DOUGLAS FIR

Control, error due to weighing $\pm 0.03\%$					<i>F. pinicola</i> 2/16/17, <i>P. versicolor</i> 3/29/17				
1061	7.29	7.29	0.00	0.00	451	8.25	7.82	0.43	5.21
1062	7.32	7.32	0.00	0.00	452	7.24	7.15	0.09	1.24
1063	7.09	7.09	0.00	0.00	453	7.08	6.92	0.16	2.26
1064	8.86	8.86	0.00	0.00	454	7.56	7.18	0.38	5.01
1065	9.08	9.07	0.01	0.11	455	7.65	7.63	0.02	0.26
1066	9.18	9.18	0.00	0.00	456	7.60	7.34	0.26	3.42
1067	8.07	8.06	0.01	0.12	457	6.99	6.94	0.05	0.71
1068	8.46	8.46	0.00	0.00	458	7.73	7.40	0.33	4.27
1069	7.21	7.21	0.00	0.00	459	7.58	7.15	0.43	5.68
1070	9.28	9.29	0.01	0.11
Average	0.03	Average	3.12
<i>Fomes pinicola</i> 2/16/17					<i>F. pinicola</i> 2/16/17, <i>L. saepiaria</i> 3/30/17				
441	7.62	7.11	0.51	6.68	461	8.32	8.05	0.27	3.24
442	8.55	8.00	0.55	5.75	463	6.43	6.35	0.08	1.24
443	7.25	7.18	0.07	0.96	464	8.02	7.77	0.25	3.12
444	7.72	7.65	0.07	0.91	465	7.62	7.50	0.12	1.58
445	8.25	7.75	0.50	6.05	466	7.39	7.04	0.35	4.75
446	7.57	7.45	0.12	1.58	467	7.78	7.56	0.22	2.83
447	8.04	7.73	0.31	3.86	469	7.06	6.67	0.39	5.50
448	8.01	7.83	0.18	2.25	470	8.16	7.76	0.40	4.90
449	8.04	7.94	0.10	1.24
450	6.84	6.81	0.03	0.44	Average	3.02

* No bacteria present after incubation.

† *Bacterium mycoides* present after incubation.

TABLE XVII (Continued)

I	II	III	IV	V	I	II	III	IV	V
Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)	Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)
<i>F. pinicola</i> 2/16/17, <i>L. saepiaria</i> & <i>P. versicolor</i> 3/30/17									
471	8.41	7.81	0.60	7.13	501	8.84	8.40	0.44	4.98
472	8.62	7.78	0.84	9.75	502	8.52	8.18	0.34	3.99
473	8.18	7.55	0.63	7.71	503	8.69	8.47	0.22	2.53
474	8.23	7.64	0.59	7.18	504	9.22	8.87	0.35	3.69
475	9.10	8.59	0.51	5.61	505	8.98	8.56	0.42	4.67
476	7.42	7.00	0.42	5.66	506†	8.21	7.71	0.50	6.09
478	7.45	6.88	0.57	7.65	507	7.77	7.31	0.46	6.28
479	8.74	8.18	0.56	6.43	508†	7.89	7.67	0.22	2.79
480	8.45	7.56	0.89	10.52	509†	7.89	7.72	0.17	2.16
Average	7.52	510	9.86	9.40	0.46	4.65
Average	Average	4.18
<i>Fomes pinicola</i> 2/16/17, <i>B. prodigiosus</i> 3/30/17									
481	9.51	8.89	0.62	6.52	521	7.87	6.96	0.91	11.56
482	9.91	9.51	0.40	4.04	522	9.49	8.12	1.37	14.45
483	10.36	9.87	0.49	4.74	523	8.39	7.22	1.17	13.98
484	9.31	8.65	0.66	7.08	524	7.56	6.65	0.91	12.01
485*	7.37	7.19	0.18	2.46	525	7.67	6.60	1.07	13.95
486	9.54	8.92	0.62	6.50	526	7.91	6.66	1.25	15.80
487*	8.83	8.36	0.47	5.32	528	7.61	6.57	1.04	13.65
488	8.35	8.01	0.34	4.07	529	9.38	8.12	1.26	13.42
489*	7.45	7.20	0.25	3.36	530	9.16	7.69	1.47	16.02
490	8.25	7.85	0.35	4.24	Average	14.98
Average	4.83	Average
<i>F. pinicola</i> 2/16/17, <i>Bact. mycoides</i> 3/31/17									
491	8.48	8.03	0.45	5.31	531	7.81	6.63	1.18	15.09
492	8.66	8.23	0.43	4.96	532*	8.67	7.36	1.31	15.13
493*	7.86	7.62	0.24	3.05	533	6.73	5.71	1.02	15.18
494	10.72	10.27	0.45	4.19	534*	8.01	6.54	1.47	18.38
495	7.63	7.45	0.18	2.36	536	7.43	6.21	1.22	16.42
496	9.26	8.71	0.55	5.92	537	7.51	6.17	1.34	17.83
497*	7.04	6.78	0.26	3.70	538*	8.58	7.00	1.58	18.45
498	10.16	9.84	0.32	3.14	539	7.44	5.98	1.46	19.61
499	7.65	7.42	0.23	3.01	540	9.53	8.15	1.38	14.47
500*	8.26	7.85	0.41	4.96	Average	16.39

* No bacteria present after incubation.

† *Bacterium mycoides* present after incubation.

TABLE XVII (Continued)

I	II	III	IV	V	I	II	III	IV	V
<i>L. saepiaria</i> 2/5/17, <i>Bact. mycoides</i> 3/31/17					<i>L. saepiaria</i> 2/5/17, <i>B. vulgatus</i> & <i>Bact. mycoides</i> 4/2/17				
541	8.70	7.35	1.35	15.51	941*	8.84	7.67	1.17	13.52
542	8.31	6.89	1.42	17.09	942*	8.40	7.21	1.19	14.19
543*	7.82	6.50	1.32	16.89	945	7.73	6.61	1.12	14.49
544*	7.79	6.62	1.17	15.01	946	8.63	7.31	1.32	15.30
545	8.59	7.03	1.56	18.17	947*	8.50	8.33	0.17	2.00
546	6.87	5.64	1.23	17.89	948	6.76	5.75	1.01	14.93
547	8.01	6.83	1.18	14.73	949	6.98	6.03	0.95	13.60
548	9.72	8.32	1.40	14.41
549*	6.62	5.72	0.90	13.59
550	7.39	6.52	0.87	11.78
Average	15.51	Average	12.57
<i>L. saepiaria</i> 2/5/17, <i>B. prodigiosus</i> & <i>Bact. mycoides</i> 3/31/17					<i>Polystictus versicolor</i> 2/5/17				
551*	8.41	6.96	1.45	17.25	592	8.69	8.68	0.01	0.12
552	7.53	6.35	1.18	15.65	593	8.64	8.63	0.01	0.12
553	8.84	7.75	1.09	14.08	599	8.75	8.73	0.02	0.23
554	7.97	6.66	1.31	16.40	600	8.69	8.67	0.02	0.23
555	7.45	6.53	0.92	12.35	603	8.17	8.16	0.01	0.12
556*	9.28	7.89	1.39	14.99	604	9.26	9.24	0.02	0.22
557	9.00	7.67	1.33	14.78	605	8.80	8.79	0.01	0.12
559	7.28	6.37	0.91	12.51	606	9.05	9.03	0.02	0.22
560†	7.89	6.81	1.09	13.71
Average	14.75	Average	0.17
<i>L. saepiaria</i> 2/5/17, <i>B. vulgaris</i> & <i>B. coli</i> 3/31/17					<i>P. versicolor</i> 2/5/17, <i>B. prodigiosus</i> 3/30/17				
570	8.47	7.49	0.98	11.55	608*	7.80	7.57	0.23	2.95
573	6.25	5.38	0.87	13.92	609	9.61	9.23	0.38	3.96
574*	7.56	6.46	1.10	14.56	611	7.27	7.15	0.12	1.65
575	9.09	7.52	1.57	17.28	612	8.13	8.01	0.12	1.47
576*	7.04	6.16	0.88	12.50	613*	7.15	7.09	0.06	0.84
577*	9.23	8.00	1.23	13.34	614	7.67	7.60	0.07	0.91
578	8.77	7.18	1.59	18.11	615	8.01	7.80	0.21	2.62
.....	616*	7.48	7.31	0.17	2.27	
.....	617	9.27	8.96	0.31	3.34	
.....	618	8.93	8.70	0.23	2.58	
Average	Average	2.26
<i>L. saepiaria</i> 2/5/17, <i>Azotobacter chroococcum</i> 4/2/17					<i>P. versicolor</i> 2/5/17, <i>Bact. mycoides</i> 3/31/17				
580	8.30	7.16	1.14	13.75	619*	7.96	7.74	0.22	2.77
581	7.22	6.11	1.11	15.38	620*	7.45	7.33	0.12	1.61
586*	7.26	6.10	1.16	15.98	621*	7.37	7.30	0.07	0.95
587*	9.37	8.86	0.51	5.09	624	7.07	6.86	0.21	2.97
588	8.31	7.11	1.20	14.45	625	9.06	8.87	0.19	2.04
589*	6.96	5.88	1.08	15.52	626	7.69	7.64	0.05	0.65
590	7.13	6.08	1.05	14.72	627	8.91	8.89	0.02	0.22
.....	628	6.62	6.57	0.05	0.75	
.....	629	8.33	8.22	0.11	1.32	
.....	630	7.13	7.07	0.06	0.84	
Average	Average	1.42

* No bacteria present after incubation.

† *Bacterium mycoides* present after incubation.

TABLE XVII (Continued)

I	II	III	IV	V		I	II	III	IV	V
Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)		Culture bottle	Weight of sawdust before decay (gms.)	Weight of sawdust after decay (gms.)	Loss in weight due to decay (gms.)	Loss due to decay (per cent)
<i>P. versicolor</i> 2/5/17, <i>Azotobacter chroococcum</i> 4/2/17										
651	7.79	7.78	0.01	0.13		951	8.88	8.75	0.13	1.46
653*	8.18	8.02	0.16	1.96		952*	7.59	7.53	0.06	0.79
654*	8.46	8.35	0.11	1.30		954†	8.59	7.54	0.05	0.58
656	8.53	7.51	0.02	0.23		955	8.09	8.03	0.06	0.74
657*	9.95	9.75	0.20	2.01		956	7.01	6.91	0.10	1.56
659	7.09	7.07	0.02	0.28		957*	7.16	7.06	0.10	1.39
660	7.29	7.25	0.04	0.55		959	7.41	7.29	0.12	1.62
.....		960	7.80	7.71	0.09	1.15
Average	0.92	Average		1.16

* No bacteria present after incubation.

† *Bacterium mycoides* present after incubation.

The average results of the foregoing series are tabulated in table XVIII.

THE GROWTH OF PURE CULTURES OF BACTERIA ON SAWDUST AND THEIR EFFECT ON THE REACTION OF THE SAWDUST EXTRACT

Due to the fact that in most of the cultures bacteria were no longer found alive at the end of the period of incubation, pure cultures of the organisms were grown on sawdust in order to determine whether or not the sawdust was toxic and the effect, if any, of the bacteria on the reaction of the extract.

Equal amounts, 5.00 gms., of sawdust were placed in 125-cc. Erlenmeyer flasks and 50 cc. of distilled water were added to each. The flasks were plugged and autoclaved for one hour and subsequently inoculated with various species of bacteria. After thirty days' incubation at room temperature transfers were made from the flasks to hard potato agar, and the reaction of the wood extract determined by titrating 5 cc. of the extracts against N/20 sodium hydroxide. Red oak, western hemlock, and Douglas fir were used in this work, with *Bacillus*

TABLE XVIII
AVERAGE LOSS PER CENT IN CULTURE SERIES A, B, C AND D

coli, *B. vulgaris*, *B. prodigiosus*, and *B. vulgatus* as the organisms.

The results of the titrations, expressed in degrees of acidity, Fuller's scale, are given in table xix. All series were run in triplicate and the following results are the average of three titrations:

TABLE XIX
THE EFFECT OF PURE CULTURES OF BACTERIA ON THE REACTION OF WOOD EXTRACTS

Organism	Red oak	Western hemlock	Douglas fir
Control.....	13.0	2.5	3.0
<i>Bacillus coli</i>	13.0	2.0	3.2
<i>Bacillus vulgaris</i>	13.5	2.5	3.0
<i>Bacillus prodigiosus</i>	13.7	3.5
<i>Bacillus vulgatus</i>	13.3	3.3

In the case of the red oak extract the end point could not be exactly determined because of the dark color of the extract which might account for the slight variations in the titrations. It is evident therefore that these organisms do not change the titratable acidity of the wood extracts to any considerable extent, if at all.

Transfers made from the cultures at the end of thirty days' incubation to hard potato agar were negative in every case, indicating that the wood extracts were either toxic to, or lacked some of, the necessary substances for continued growth of the organisms.

DISCUSSION OF RESULTS

SERIES A: RED OAK

The rate of decay of red oak was comparatively slow with the three fungi used. *Lenzites saepiaria* caused the most rapid loss in weight in the culture bottles. In cultures first inoculated with *Fomes pinicola* and subsequently with *Polystictus versicolor* and *Lenzites saepiaria* the rate of decay was greater than that caused by any of the fungi growing separately and indeed greater than the combined rate of the three. In all cases the rates of decay caused by the fungi alone were less than those to which cultures of bacteria were added. With *Fomes pinicola* the highest rate of decay was produced in cul-

ture bottles to which *Bacterium mycoides* was added; with *Lenzites saepiaria* the highest rate was in the culture bottles to which *Bacillus prodigiosus* was added; and with *Polystictus versicolor* in those to which *Bacillus vulgaris* and *B. coli* were added. The only bacteria found in the culture bottles at the end of the period of incubation were *Bacillus vulgatus* and *B. coli*.

SERIES B: WHITE ASH

The highest rate of decay caused by the fungi was that caused by *Lenzites saepiaria*. Cultures of *Polystictus versicolor* to which bacteria were added lost the most weight due to decay of any of the cultures of the entire series. Culture bottles first inoculated with *Fomes pinicola* and subsequently with *Lenzites saepiaria*, *Polystictus versicolor*, or both, lost less weight than those inoculated with *Lenzites saepiaria* or *Polystictus versicolor* alone. This is quite different from the results obtained with red oak. The addition of bacteria to cultures of *Fomes pinicola* caused no change in the rate of decay. In the case of *Polystictus versicolor* culture bottles to which bacteria were subsequently added lost more weight than did pure cultures of the fungus. The greatest loss in weight is found in cultures of *Polystictus versicolor* to which *Bacillus prodigiosus* and *Bacterium mycoides* were added. *Bacillus vulgatus* and *Bacterium mycoides* were the only bacteria found in the culture bottles at the end of the period of incubation.

SERIES C: WESTERN HEMLOCK

Lenzites saepiaria caused the highest rate of decay on western hemlock of any of the fungi used. Cultures first inoculated with *Fomes pinicola* and subsequently with *Polystictus versicolor* lost no more weight than those inoculated with *Fomes pinicola* alone. In *Fomes pinicola* cultures subsequently inoculated with *Lenzites saepiaria* the loss in weight was practically identical to that lost by pure cultures of *Lenzites saepiaria*. When *Fomes pinicola* cultures were subsequently inoculated with both *Lenzites saepiaria* and *Polystictus versicolor* the loss in weight was also practically the same as that caused by pure cultures of *Lenzites saepiaria*. Cul-

tures of *Polystictus versicolor* to which bacteria were added, in all cases lost from two to three times more weight than was lost by pure cultures of the fungus. *Bacterium mycoides* was the only bacterium found in the culture bottles at the end of the period of incubation.

SERIES D: DOUGLAS FIR

Lenzites saepiaria caused the most rapid rate of decay of Douglas fir. The addition of bacteria to cultures of *Fomes pinicola* and *Lenzites saepiaria* caused no apparent change in the rate of decay. Cultures of *Polystictus versicolor* to which bacteria were added lost from five to ten times as much weight as did cultures of the fungus alone. *Bacterium mycoides* was the only bacterium found in the cultures at the end of the period of incubation.

SUMMARY

The experimental results obtained may be briefly summarized as follows:

1. When wood is sterilized by autoclaving it undergoes certain changes which must be considered when using wood for experimental purposes with wood-decaying fungi. Among these changes are: (a) a change in color; (b) an increase in the amount of reducing substances in the extract; (c) an increase in the acidity of the extract; (d) an increase in the hydrogen ion concentration of the extract; (e) a change in resistance towards decay.
2. Cellulose-dissolving bacteria play no important part in the decay of wood under natural conditions.
3. The results from the decaying experiments tend to indicate that the rate of decay may be materially increased by the presence of the ordinary saprophytic bacteria.
4. The influence of bacteria on fungi with reference to the rate of decay induced by the fungi varies with the different fungi on different woods.
5. Further experimentation along this line is essential.

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